

**BEST AVAILABLE COPY****REMARKS**

Reconsideration of the application, as amended, is respectfully requested.

Claims 2 and 6 have been amended to eliminate an informality as suggested by the Examiner.

US 5 607 712 (Bourne) discloses a process for improving the firmness of vegetables which are frozen before canning comprising the steps of blanching a vegetable under two different conditions, freezing the blanched vegetable, storing the frozen vegetable, and further successive steps relating to canning the vegetable.

The description indicates that the vegetable can be "frozen quickly" (see column 4 (line 14) and that the frozen vegetable is "usually stored ... at or below 0°F" (see column 4 (lines 21-23)).

Vegetables which are frozen in the conventional manner (i.e. quick-frozen) show, after being cooked from their frozen state, a mushy and poor in-mouth texture (see the present examples). The undersigned has been informed that this is thought to be due to the formation of ice crystals damaging the vegetable cell walls (see page 363 of Fennema et al, enclosed, which the undersigned has enlarged—the undersigned is attempting to obtain clearer copies of Fennema et al. and will furnish them as soon as they are obtained). Fennema et al. is "Low Temperature Preservation of Foods and Living Matter," Marcel Dekker, NY 1973, pp352-385. In particular "several studies with light and electron microscopes have provided support for the supposition that extracellular ice crystals separate and rupture cell walls by accumulation of ice in the middle lamellae" (see page 363 of Fennema et al.) See instant Example 1 and Figures 4-7.

The inventors have devised a process for the production of a frozen vegetable or part which includes the step of under-cooling said vegetable or part thereof to a core temperature of less than or equal to -5°C. The present specification explains that this step "ensures that enough

heat has been removed from the material to allow rapid and uniform ice formation in the freezing step (iii) and thereby provide a significant reduction in extracellular ice formation" (see

page 9 (lines 2-6)). The instant specification further discloses: "It has been shown that merely reducing the core to -1 or -2°C without further under-cooling is not sufficient for the rapid initiation of freezing needed for the desired reduction in extracellular ice..." (see page 9 (lines 8-12)).

The Office points to no teaching by US 5 607 712 (Bourne) of undercooling to a core temperature of less than or equal to -5°C and thus applicants submit that the subject matter of claim 1 is inventive over US 5 607 712 (Bourne).

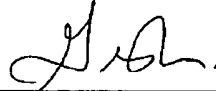
US 3 136 642 (Frane et al), discloses a process for improving the thawing consistency of fruit and vegetable salad for withstanding "the stresses of a quick-freezing and thawing operation" (see column 2 (line 1)). Examples 1 and 2 of Frane et al also disclose that the freezing process is quick-freezing by direct immersion of the subject vegetable pieces in liquid dichlorodifluoromethane (boiling point of -21°F) and liquid octofluorocyclobutane (boiling point of -20°F) respectively. The Office again points to no teaching of undercooling to a core temperature of less than or equal to -5°C.

As to Bengtsson, et al., the Office again points to no teaching of undercooling to a core temperature of less than or equal to -5°C. Upon review of the file, applicants have been unable to identify a reference which is listed on Forms PTO-1449 or PTO-892 with that name. Therefore, it would be appreciated if the Office would ensure that Bengtsson et al. is cited of reference and inform applicants of the document type and number.

In view of the foregoing, it is respectfully requested that the application, as amended,

be allowed.

Respectfully submitted,



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**FOOD SCIENCE**  
A Series of Monographs

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**LOW-TEMPERATURE PRESERVATION  
OF FOODS AND LIVING MATTER**

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CHAPTER 7  
CHARACTERISTICS OF FOOD PHYTOCHEMICALS  
AND THEIR BEHAVIOR DURING FREEZE-PRESERVATION

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Plants and vegetables as human food (food phytochemicals) are in demand throughout the year. Since the production of these raw products is seasonal in most parts of the world and where harvested edible plant products deteriorate rapidly during storage at ambient temperatures, some means of preservation is essential for long-term storage. Ideally, the native structure and chemical composition of recently harvested fruits and vegetables should be preserved until they are utilized or converted into food products through processing. Such an idealistic requirement would involve immobilization of viable enzymes, complete cessation and stabilization of localized water, maintenance of natural barriers against microbial invasion, and at least partial maintenance of microcirculation.

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Enzymes and phytochemicals, which are primarily responsible for quality deterioration of harvested plant tissues during storage, can be immobilized by thermal energy but extensively structural and chemical changes of the native tissues are inevitable and consequently undesirable from a quality standpoint. For example, the heat treatment of strawberries blanches the delicate flavor of the raw product, reduces the viscosity of the cells, and disrupts the middle lamellae between cells [187].

The approach to maintaining an acceptable quality of raw fruits and vegetables for an extended storage period is to reduce the respiration and transpiration rates by lowering the temperature of the product to near the freezing point, and by controlling the  $O_2$ ,  $CO_2$ , and water contents of the environment [18, 20, 207, 208]. Applied in particular have been stored under controlled-atmospheric conditions for periods up to about one year with only slight to moderate damage to the eating quality. Storage of fresh fruits and vegetables such as carrots and potatoes can be prolonged for many months without significant quality deterioration simply by decreasing the temperature to about 7° or lower. These commodities have relatively low rates of respiration and have natural barrier cells which form a protective coating resistant to external attack. However, the chemical composition and quality of many fruits and vegetables are changed even when low-microfreezing temperatures and low levels of oxygen are maintained. Peas, corn, green beans, and asparagus undergo rapid losses of sugars after harvesting because the high rates of respiration cannot be depressed sufficiently by lowering the temperature or controlling the  $O_2$  and  $CO_2$  in the environment. For prolonged storage of food phytochemicals, a preservation method such as freezing is essential.

When perishable plant tissues are frozen and stored under proper conditions, the natural color, flavor, and nutrients are well-retained for several years. However, delicate tissues of some food phytochemicals, particularly onions, may be damaged extensively by ice crystals. Such crystals may impair the natural quality of the stored product because of a reduction of cell turgidity and a loss of cell fluids or sap (dew-matted). Addition of sugar to plant tissues prior to freezing has been reported to lessen this type of damage. Previous characterization of native fruit tissues can be well-retained by freezing and maintaining small intracellular ice crystals through very rapid freezing, low storage temperatures, and rapid thawing.

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Several excellent reviews on the preservation of fruits and vegetables by freezing have been published within the last ten years [29, 36, 37, 42, 49, 57, 70, 83-85, 87, 88].

## 7-1 CLASSIFICATION AND STRUCTURE OF EDIBLE PLANT TISSUES

Plant tissues, in either the raw or processed state, are consumed by mankind the world over. However, only a specific part of a plant body is generally selected for consumption. Through the centuries, man has selected plant parts on the basis of their organoleptic appeal. Fibrous plant tissues are frequently unacceptable to the consumer of ready eaten food products and are difficult to masticate and swallow. Plant tissues with a high degree of bitterness are usually rejected as inedible. On the other hand, fleshy plant tissues with a juicy aroma or a pleasant heat-induced odor (formed in vegetables during cooking) are considered organoleptically acceptable. The fleshy tissues of the potato plant are used as a food commodity whereas the fibrous stems, leaves, and roots are discarded as unpalatable for human consumption. Upon cooking the potato tuber, the tissue becomes soft enough to be palatable and a desirable protein source develops.

Food phytochemicals are classified broadly as fruits, vegetables, cereals, and nuts. Only fruits and vegetables are discussed in this chapter. Fruit, from a strictly botanical standpoint, refers to the mature ovary and other selected flower structures. Components of a fruit include seeds, an ovary wall (pericarp), and derivatives of the receptacle, petals, sepals, and stamens. This fruit from a botanical standpoint includes not only edible fleshy fruits but also dry kernels of the grain family and mature tree nuts. With respect to food science, a fruit is generally regarded as a juicy fleshy ovary which has a high moisture content, distinctive aroma, and is either naturally sweet or sweetened with sugars [29]. In most instances, fruits contain a considerable amount of organic acids which are responsible for their low pH values. Fruits are commonly used as dessert foods or as ingredients for salads.

Vegetables include roots, stems, shoots, modified stems (bulbs and tubers), leaves, and immature seeds. Typical characteristics of raw vegetables are a low concentration of sugars, little if any aroma, high

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cellulose content, and a relatively firm flesh. In many cases, a desirable aroma arises during the cooking of vegetables.

Damage to fruits and vegetables tissues during freezing and storage can be attributed to alterations of the cellular lamella, cell walls, and cell contents. The development of extracellular ice crystals may cause cell separation in the cellular lamella regions, cell wall rupture, and cell shrinkage. Intracellular ice crystal formation by rapid freezing of plant tissues may bring about death of living cells but with a minimum in other undesirable ice maintaining the organoleptic quality of food phytochemicals.

Before a critical discussion on the cryobiology of edible plant tissues can be developed satisfactorily, the structure of the plant cell must be described. Fruit and vegetable tissues are made up of a variety of cells differing structurally and functionally. Edible plant tissues consist for the most part of parenchyma cells [117]. Parenchyma is often referred to as ground tissue which vascular tissues are embedded [27]. Parenchyma tissues in developing plants consist of living cells involved in activities such as photosynthesis, respiration, expansion, and secretion. Mature parenchyma tissue for human food may contain considerable amounts of sugar, starch, and/or protein as storage compounds. The diameter of a parenchyma cell ranges from about 20 to 300  $\mu$  [26]. In the majority of mature many seeded, parenchyma cells have a mean length of about 119  $\mu$  [82]. The shape of parenchyma cells is generally described as polyhedral and each cell has an average of 14 faces [91, 92]. If parenchyma tissue is made up of cells with a variety of shapes, the small cells have fewer than 14 faces and the large cells have more than 14.

All cells of higher plants are made up of a cellulose cell wall and a protoplasm (Fig. 7-1). Each cell has a thin primary wall and, in addition, a secondary wall which is layered inwardly upon the primary wall. The parenchyma cells of many types of fruits and vegetables have only primary walls. However, some vegetable tissues (e.g., peas, lima beans) possess cells with thick secondary walls. The primary and secondary walls are made up of (1) fibrous substances and (2) ground compounds as the hemicellulose [22]. The major fibrous substance in cell walls of edible



Fig. 7-1. Parenchyma cell of a tomato fruit. CW, cell wall; V, vacuole; PM, plasmalemma; Y, amyloplast; N, nucleus; P, plastid; M, mitochondrion; RW, rough-surfaced endoplasmic reticulum; G, Golgi body. (From Mohr and Castles, Ref. [26]; courtesy of Academic Press.)

cytic and vegetable to cellulose (6-1, 4-pyranose). Cellulose molecules are unbranched polymers consisting of 1000 or more glucose residues. The matrix of the cell wall is made up of hemicelluloses, pectic substances, proteins and lipids.

According to Butterfield and Hayley [24], the average composition of primary cell walls on a dry weight basis is: hemicelluloses, 23%; cellulose, 20%; lipids, 7%; pectic substances, 5%; and proteins, 24. Lipids may also be present in the primary wall of some plant cells. In the secondary walls, cellulose is the major component [25]. Water in the cell walls is bound largely to the cellulose where it is bound to hydrophilic compounds such as pectin and hemicelluloses.

Cavities are frequently observed in cell walls, particularly in secondary walls [27]. A cavity in the secondary wall is called a pit. A depression in the primary wall has been termed a primary-wall field [125]. Pits are generally arranged in pairs, one being opposite to another in an adjacent cell wall (Fig. 7-2). A pit membrane, made up of

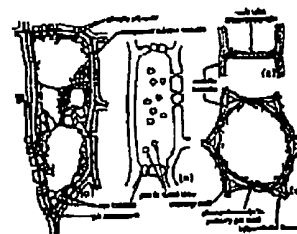


Fig. 7-2. Pits and plasmodesmata in cell walls. (a) and (b), cells with secondary walls; (c) and (d), parenchyma cells without secondary walls. (From Jan, Ref. [27]; courtesy of John Wiley and Sons.)

the thin primary walls with a middle lamella between them, separates adjacent pits. Several alveoli (plasmodesmata) extend from the protoplast of one cell into an adjacent cell through the pit membrane. Plasmodesmata are considered to be channels for the flow of nutrients between cells [27].

Cellulose molecules in cell walls of higher plants are not randomly distributed but are oriented longitudinally in the form of bundles. Up to about 100 molecules of cellulose are combined through hydrogen bonding to form ribbon-like elementary fibrils. Cellulose molecules in the elementary fibrils are aligned parallel to their long axis in such a manner that some crystalline regions are formed [27]. These crystalline regions, called microfibrils, are made up of cell walls and with their glucose residues [27]. Microfibrils within the primary cell walls have diameters of 20-25 Å, whereas microfibrils within secondary walls have diameters of 50-60 Å [106]. As much as 50 to 60% of the cellulose in the cell walls of plants is in a crystalline state. The microfibrils in the elementary fibrils are separated longitudinally by amorphous regions. The microfibrils in cell walls are resistant to enzymic hydrolysis and they absorb very little water [106]. On the other hand, the amorphous regions which contain relatively few cellulose-cellulose hydrogen bonds, possess large

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members of hydrogen bonds which correspond with water molecules. The micro-morphous nature can be changed by heating and by dehydration [102].

The elementary fibrils in cell walls are aggregated (with their long axes parallel to each other) in the form of strands termed microfibrils. The microfibrils, with diameters between 100 and 300 Å are oriented in a variety of patterns. Generally speaking, microfibrils in primary walls have a random or nonparallel orientation while those in the secondary layer of a secondary wall are arranged parallel to each other [82].

In intact tissues, adjoining cells are held together by amorphous polymers in an intercellular layer called the middle lamella. The principal polymers are pectic substances such as protopectin and pectin [27, 31, 39, 100]. Calcium ions play an important role in cell cohesion by bridging the pectic polymers in the middle lamella [10]. Van Rure et al. [16] have demonstrated the relationship between the chemical nature of pectic substances in the middle lamella and the firmness of carrot green tissue. Separation of plant cells can be brought about by the addition of chelating agents [18, 28, 84].

The cells of fleshy succulent tissues of fruit often are not in perfect contact, thus giving rise to intercellular gas spaces. In mature apple tissues, Rees [90] calculated that 20 to 30% of the total tissue volume was made up of intercellular gas spaces, some being as long as 100 µ. Water and Smalley [117] reported that mature peach tissues contained as much as 15 vol % intercellular space. On the other hand, the intercellular volumes in the potato tuber and dairy bean tubers are insignificant.

All protoplasts of higher plant cells are separated from the cell walls by a cell membrane, the plasmalemma. The plasmalemma, about 100 Å thick, is a unit membrane with a granular structure [74]. The structure has been investigated by Fry-Speckling and Rauterbach [32] as a dynamic bimolecular layer of globular lipoproteins complexes with diameters of 20 to 30 Å. The functions of the plasmalemma are to control the passage of water and solutes in and out of the cell and to catalyze the degradation of specific substances.

The permeability is composed of (1) compounds components such as cytoplasm, nucleus, plasma, and other organelles, and (2) membrane components such as vitamins, enzymes, growth regulators, and all enzymes.

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The cytoplasm is a continuous viscous fluid or gel acting as a matrix for organelles and other particulate matter [27]. The volume in solution are proteins, amino, polyelectrolytes, and ionogenic ions. In unexcited nerve cells, the cytoplasm is confined to a thin layer near to the inside surface of the cell wall. In fully actively dividing cells, the cytoplasm samples most of the cell volume. The water content of active cytoplasm is about 85 to 90% [28].

In many plant tissues, small colored or colorless bodies called plastids are distributed throughout the cytoplasm [27]. The colored plastids are classified as chloroplasts (green) and chromoplasts (yellow, orange, and red); chromoplasts are colorless plastids. All green plant tissues possess chloroplasts in their cells. The chloroplasts of higher plants are usually disk-shaped bodies with diameters of about 4 to 6 µ. The green granules within the chloroplasts, consist most of the chlorophyll in green cells. Chromoplasts occur in a variety of shapes such as elongated, lobed, and spherical, and all of them contain carotenoids. Amyloplasts are leucoplasts which have the sole function of synthesizing starch.

Myo-epithelial cells possess a large central vacuole which is enclosed by a thin membrane called the tonoplast [27]. Young cells often contain two or more vacuoles. The tonoplast is a unit membrane with a structure similar to that of the plasmalemma [79]. The mechanical strength of the tonoplast, however, is greater than that of the plasmalemma. Water is the major constituent of the vacuole often amounting to levels as high as 90% [103]. The solution in the vacuole fluid consists of sugars, organic acids, salts, anthocyanins, and proteins. The turgidity of cells is associated with the concentration of vacuolar solution and the differential permeability of the tonoplast to ions and water.

## 7-2 COOLING, SUPERCOOLING, AND FREEZING OF PLANT TISSUES

The initial stage of the freezing process involves cooling the product to a temperature just above its freezing point. Rapid cooling of plant tissues can lead to an increase in membrane permeability and loss of turgor [47]. Such injury is known as thermal shock. This and

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Partridge [17] found that the cells in the pericycle of the plant *Citrus* lost their turgor upon short exposure to temperatures in the vicinity of  $0^{\circ}$ . According to these investigations, the electrical conductivity of the tissue increased within the first few hours of exposure to cold temperatures, and this increase was mainly due to the permeability of the membrane and/or cell wall to water and ions. In the other hand, cited at [1], [12] were unable to demonstrate chemical shock in chilled peas which were rapidly cooled to  $0^{\circ}$ . Thus it is possible that the thermal shock of edible plant tissues could contribute to the deterioration of quality that fruits and vegetables undergo during the freezing process.

Supercooling of higher plant tissues is generally confined to a few degrees below the freezing point [10, 14]. Henderberty [16] reported that certain fruits could be supercooled to about  $-5^{\circ}$  and Henderberty and Harvill [12] found that some fruits supercooled in a constant cold to supercooling to  $-9^{\circ}$ . Under normal commercial freezing conditions, the duration of supercooling in fruits and vegetable tissues is usually brief, i.e., a matter of seconds [17, 20, 23, 24, 25]. However, under special conditions, supercooling of plant tissues can be extended for long periods of time. For example, chilled pea shoots can be held in a supercooled state at  $-4^{\circ}$  if the shoots are coated with paraffin to prevent surface nucleation of ice [15].

External water, surrounding metabolically active cells, behaves before the freezing point of the protoplast [70]. Even when ice is present on the cell wall surfaces, the protoplast can remain in a supercooled state than freezing temperatures are as low as  $-12^{\circ}$ . Solid [9, 16] reported that some of the pericycle cells of the pericycle of cabbage tissues at  $-12^{\circ}$  underwent intracellular freezing even when the tissue was sealed with ice. According to Chambers and Hinde [13], the living protoplasts of epidermal cells from scales of a red onion can also be supercooled to  $-9^{\circ}$  even though ice crystals formed on the surfaces of the cells. When the freezing temperature was dropped to  $-10^{\circ}$ , the protoplasts were destroyed and the vacuole was not evident immediately after freezing. However, after 1 hr of further storage at  $-10^{\circ}$ , the number of ice crystals

increased in the protoplasts and the vacuole separated into smaller, densely pigmented vesicles with intact membranes surrounding them. Presumably on further nucleation previous nucleation of intracellular ice is mainly an initial nucleation process nucleation of intracellular ice in plant tissues at temperatures above  $-5^{\circ}$  [10, 16]. As temperatures below  $-5^{\circ}$ , the critical radius of ice may be smaller than the radius of the pores in the plasma membrane thereby enabling intracellular ice to grow through the intact plasma membrane and promote formation of intracellular ice.

Since intracellular supercooled water has a higher vapor pressure and free energy than external ice at the same temperature, the intracellular water moves through the cell walls and contributes to the growth of extracellular ice crystals [70]. Outward flow of intracellular water will continue as long as the internal water remains unfrozen and has a vapor pressure higher than the external ice. Transport of supercooled intracellular water cannot occur when plant tissues are frozen slowly and is stored at a relatively high subfreezing temperature.

The freezing points of soluble fruit and vegetable tissues are determined by the concentration of water-soluble solutes (soluble solids) especially sugars, salts, and acids [8]. Figure 7-3 shows the most predominant soluble solids in food phytochemicals. The total soluble solids in fruits and vegetables generally range between 4 and 24% [11]. As shown in Fig. 7-3, the freezing points of fruit and vegetable tissues are directly related to their soluble solids contents.

As the temperature of tissue is decreased below the freezing point, its ice content increases steadily and water-soluble solutes and suspended matter become increasingly more concentrated in the remaining unfrozen aqueous phase. The amount of ice in plant tissues at a given subfreezing temperature is dependent on the initial content of soluble solids. Table 7-1 summarizes the ice contents of a wide variety of fruits and vegetables at temperatures between  $-1^{\circ}$  and  $-20^{\circ}$  and some data for strawberries, pears, and sweet cherries are shown in Fig. 7-4. In strawberries at  $-5^{\circ}$ , about 80% of the total water is frozen, whereas in sweet cherries, with one and one-half times more soluble solids, only 40% of the total water is frozen. When the temperature is dropped to  $-10^{\circ}$ , 80% or more of the water is frozen in all three plant products.

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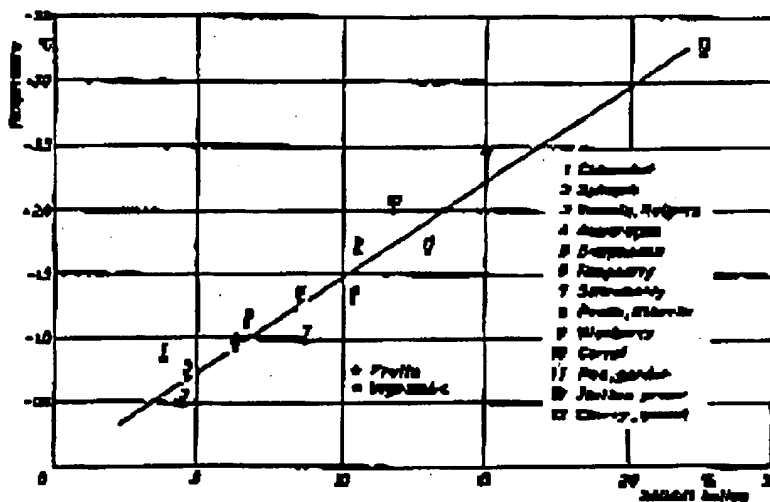


Fig. 7-3. Freezing points of some fruits and vegetables in relation to their soluble solids content. (From Gutschmidt, Ref. [42]; courtesy of Pergamon Press.)

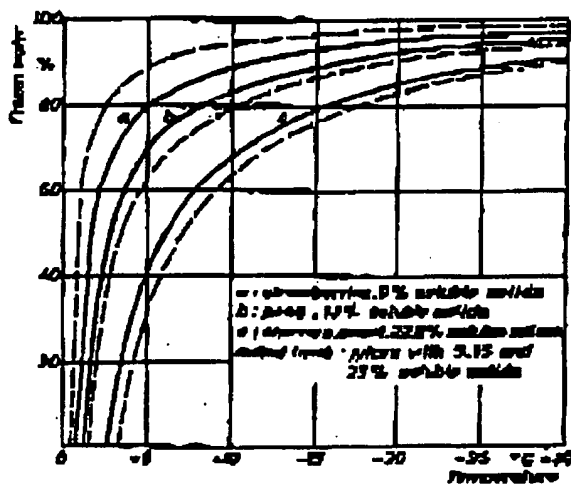


Fig. 7-4. Amount of ice in some food phytochemicals at various sub-freezing temperatures (From Gutschmidt, Ref. [42]; courtesy of Pergamon Press.)

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7-3 STRUCTURAL AND TEXTURAL DETERIORATION  
OF PLANT TISSUES DURING FREEZING

## 7-3.1 Microstructural Alterations

The formation of ice crystals in plant tissues may lead to irreversible damage of the cell walls, middle lamellae, and protoplasts. With such structural damage, textural properties of a thawed food phytoystem may be much inferior to those of the unfrozen tissue. For example, the firmness of apple tissue is reduced considerably by freezing regardless of the rate of ice formation [101]. Several studies have shown that the extent of textural damage (softening of tissue) is related to the degree of tissue disruption [12, 91, 101, 118]. The type and extent of cryodamage to plant tissue is dependent on ice crystal locality and size, and these in turn are governed by the rate of freezing and conditions of frozen storage.

When plant tissue is frozen slowly, ice crystals are located predominantly in extracellular regions [12, 41, 76, 101, 120]. Intercellular spaces are probably the sites where ice crystallization is first initiated [120]. These spaces undoubtedly contain water vapor which condenses on the cell walls as small water droplets and subsequently converts to microscopic ice crystals at freezing temperatures. During exclusive extracellular crystallization, a few large crystals are formed, water is dislocated from the cell and shrinkage of the cell occurs. Sahai [97] found that water withdrawn from plant cells during extracellular freezing took place at temperatures as low as  $-10^{\circ}$ . Such changes are undoubtedly responsible for alterations in the tissue ultrastructure.

With the loss of intracellular water during extracellular freezing, the concentration of inorganic salts increases in the protoplast, perhaps to a level sufficient to precipitate proteins [77]. Levitt [67] postulated that death of plant cells occurs by an irreversible protein-protein interaction involving a sulphydryl-disulfide interchange.

Several studies with light and electron microscopes have provided support for the supposition that extracellular ice crystals separate and rupture cell walls by accumulation of ice in the middle lamellae [12, 47, 69, 77, 101, 120]. Woodroof [120], using peaches, strawberries, and raspberries, noted microscopically that the cell walls were punctured by

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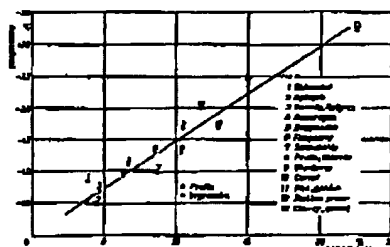


Fig. 7-3. Freezing points of some fruits and vegetables in relation to their soluble solids content. (From Gutschalk, Ref. [42]; courtesy of Pergamon Press.)

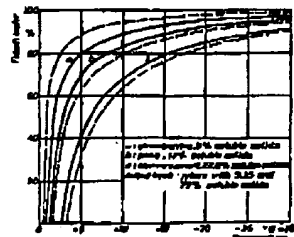


Fig. 7-4. Amount of ice in three fruit phytochemicals at various sub-freezing temperatures. (From Gutschalk, Ref. [42]; courtesy of Pergamon Press.)

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## 3-3 STRUCTURAL AND TEXTURAL DETERIORATION OF PLANT TISSUES DURING FREEZING

## 3-3.1 Microstructural Alterations

The formation of ice crystals in plant tissues may lead to irreversible damage of the cell walls, middle lamellae, and protoplasts. With such structural damage, textural properties of a frozen food phytochemical may be much inferior to those of the unfrozen tissue. For example, the firmness of apple tissue is reduced considerably by freezing regardless of the rate of ice formation [101]. Several studies have shown that the extent of structural damage (softening of tissue) is related to the degree of tissue disruption [12, 21, 301, 128]. The type and extent of cryodamage to plant tissue is dependent on the crystal locality and size, and these in turn are governed by the rate of freezing and conditions of frozen storage.

When plant tissue is frozen slowly, ice crystals are located predominantly in extracellular spaces [12, 41, 76, 101, 128]. Intracellular spaces are probably the sites where ice crystallization is first initiated [128]. These spaces undoubtedly contain water vapor which condenses on the cell walls as small water droplets and subsequently converts to microcrystalline ice crystals at freezing temperatures. During extensive extracellular crystallization, a few large crystals are formed, which in addition to the cell walls and cytoplasm of the cell rupture. Snow [197] found that water withdrawn from plant cells during extracellular freezing took place at temperatures as low as  $-30^{\circ}\text{C}$ . Such changes are undoubtedly responsible for alterations in the tissue ultrastructure.

With the loss of intracellular water during extracellular freezing, the concentration of solutes in the protoplasts increases. Perhaps to a level sufficient to precipitate proteins [77]. Lovett [67] postulated that much of plant cell injury by an irreversible protein-protein interaction involving a carbohydrate-alanine interchange.

Several studies with light and electron microscopes have provided support for the suggestion that extracellular ice crystals separate and rupture cell walls by accumulation of ice in the middle lamellae [12, 47, 69, 77, 101, 128]. Woodruff [100], using patches, microtomes, and microprojectors, noted microscopically that the cell walls were punctured by

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ICE RECRYSTALLIZATION OF FRUITS

Ice crystals during the freezing process. Dendrocytes freeze slowly in air and more than 90% of the cell walls in a structure of cells, but with rapid freezing, cell wall damage was only 10%. When Stirling [10] subjected apple tissue to slow freezing conditions, he observed extensive cell separation, cell compression, and rupture of cell walls. In blanched green beans, the fragile cell walls of the inner parenchyma are disrupted by ice crystals even with moderately rapid freezing [12]. With very slow freezing (20 min or longer), the thick-walled cell in the outer parenchyma of the blanched bean pod were often separated from each other but breakage of cell walls was not evident. Freezing damage to blanched bean cells is noticeable even in the thawed product.

Since light microscopy cannot be used to evaluate cryodamage to the fine structure of parenchyma, Mohr and Stain [7] performed a fluorescence technique so that the undistorted and frozen parenchyma tissues of tomato fruit could be examined by electron microscopy. Light and electron micrographs of freeze-dissociated tomato tissues stained clearly at rates less than 10° per min revealed enlargement of intercellular spaces, breakage of some cell walls, and penetration of protoplasm to the degree that it occupied only a small fraction of the total volume of each cell. Rupture of the cell walls appeared to occur in the middle lamella region. According to Mohr and Stain [7], cytoplasmic protrusions, cytoplasm, oleiferous globules, Golgi bodies, mitochondria, and plastid lamellae were not retained in their original places when tomato parenchyma was frozen slowly. Microscopic studies by Hunter and Harvey [8] and Tasse and Brown [9] have shown that vascular bundles as well as parenchyma cells are damaged during the slow freezing of asparagus.

When the rate of freezing of plant tissue is greater than about 10° per min, parenchyma ice crystals predominate [14, 17]. Electron micrographs of freeze-stained tissues of rapidly frozen tomato parenchyma revealed the presence of very small ice crystals inside and outside the cells as well as in the cell walls [77]. When parenchyma of tomato fruit was frozen at rates greater than 10° per min, Mohr and Stain [7] found that intercellular spaces did not become enlarged, cell walls did not separate or fracture, and the protoplasm remained close to the cell wall along with movement from the cells was restricted. According to Stirling [10], cryodamage to the cell walls of apple tissue frozen in liquid nitrogen was not apparent by light microscopy. Similarly, the cell

structure of liquid-nitrogen frozen (LNF) green beans appeared to be about the same as that of unfrozen beans [119].

Indicatively rapid freezing (1 to 5 min) of rapidly frozen tomato parenchyma causes the least damage to the protoplasm but extensive disruption is still observed [77]. However, the plasmalemma and lamellar system of the plastids often remain reasonably intact and sometimes the cytoplasmic area are disrupted during the rapid freezing-modestly-rapid thawing regime. Maintenance of structural integrity of plant material in highly viscous media is important for preservation of hydrostatic pressure within the cells, and for the prevention of drip and tissue softening.

Ice crystals in frozen plant tissues usually increase in size during frozen storage and in the early stages of thawing. Such a phenomenon is called recrystallization, and it can have a profound damaging effect on tissue cells and can lead to changes in the microstructure and a loss of tissue firmness. Luyck and Gies [68] observed recrystallization in rapidly frozen epididymal cells of the onion between -4 and -6°. In 1913, 1927, 1930, 1931, 1932, 1933, 1934, 1935, 1936, 1937, 1938, 1939, 1940, 1941, 1942, 1943, 1944, 1945, 1946, 1947, 1948, 1949, 1950, 1951, 1952, 1953, 1954, 1955, 1956, 1957, 1958, 1959, 1960, 1961, 1962, 1963, 1964, 1965, 1966, 1967, 1968, 1969, 1970, 1971, 1972, 1973, 1974, 1975, 1976, 1977, 1978, 1979, 1980, 1981, 1982, 1983, 1984, 1985, 1986, 1987, 1988, 1989, 1990, 1991, 1992, 1993, 1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021, 2022, 2023, 2024, 2025, 2026, 2027, 2028, 2029, 2030, 2031, 2032, 2033, 2034, 2035, 2036, 2037, 2038, 2039, 2040, 2041, 2042, 2043, 2044, 2045, 2046, 2047, 2048, 2049, 2050, 2051, 2052, 2053, 2054, 2055, 2056, 2057, 2058, 2059, 2060, 2061, 2062, 2063, 2064, 2065, 2066, 2067, 2068, 2069, 2070, 2071, 2072, 2073, 2074, 2075, 2076, 2077, 2078, 2079, 2080, 2081, 2082, 2083, 2084, 2085, 2086, 2087, 2088, 2089, 2090, 2091, 2092, 2093, 2094, 2095, 2096, 2097, 2098, 2099, 2100, 2101, 2102, 2103, 2104, 2105, 2106, 2107, 2108, 2109, 2110, 2111, 2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119, 2120, 2121, 2122, 2123, 2124, 2125, 2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133, 2134, 2135, 2136, 2137, 2138, 2139, 2140, 2141, 2142, 2143, 2144, 2145, 2146, 2147, 2148, 2149, 2150, 2151, 2152, 2153, 2154, 2155, 2156, 2157, 2158, 2159, 2160, 2161, 2162, 2163, 2164, 2165, 2166, 2167, 2168, 2169, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185, 2186, 2187, 2188, 2189, 2190, 2191, 2192, 2193, 2194, 2195, 2196, 2197, 2198, 2199, 2200, 2201, 2202, 2203, 2204, 2205, 2206, 2207, 2208, 2209, 2210, 2211, 2212, 2213, 2214, 2215, 2216, 2217, 2218, 2219, 2220, 2221, 2222, 2223, 2224, 2225, 2226, 2227, 2228, 2229, 2230, 2231, 2232, 2233, 2234, 2235, 2236, 2237, 2238, 2239, 2240, 2241, 2242, 2243, 2244, 2245, 2246, 2247, 2248, 2249, 2250, 2251, 2252, 2253, 2254, 2255, 2256, 2257, 2258, 2259, 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3256, 3257, 3258, 3259, 3260, 3261, 3262, 3263, 3264, 3265, 3266, 3267, 3268, 3269, 3270, 3271, 3272, 3273, 3274, 3275, 3276, 3277, 3278, 3279, 3280, 3281, 3282, 3283, 3284, 3285, 3286, 3287, 3288, 3289, 3290, 3291, 3292, 3293, 3294, 3295, 3296, 3297, 3298, 3299, 3300, 3301, 3302, 3303, 3304, 3305, 3306, 3307, 3308, 3309, 3310, 3311, 3312, 3313, 3314, 3315, 3316, 3317, 3318, 3319, 3320, 3321, 3322, 3323, 3324, 3325, 3326, 3327, 3328, 3329, 3330, 3331, 3332, 3333, 3334, 3335, 3336, 3337, 3338, 3339, 3340, 3341, 3342, 3343, 3344, 3345, 3346, 3347, 3348, 3349, 3350, 3351, 3352, 3353, 3354, 3355, 3356, 3357, 3358, 3359, 3360, 3361, 3362, 3363, 3364, 3365, 3366, 3367, 3368, 3369, 3370, 3371, 3372, 3373, 3374, 3375, 3376, 3377, 3378, 3379, 3380, 3381, 3382, 3383, 3384, 3385, 3386, 3387, 3388, 3389, 3390, 3391, 3392, 3393, 3394, 3395, 3396, 3397, 3398, 3399, 3400, 3401, 3402, 3403, 3404, 3405, 3406, 3407, 3408, 3409, 3410, 3411, 3412, 3413, 3414, 3415, 3416, 3417, 3418, 3419, 3420, 3421, 3422, 3423, 3424, 3425, 3426, 3427, 3428, 3429, 3430, 3431, 3432, 3433, 3434, 3435, 3436, 3437, 3438, 3439, 3440, 3441, 3442, 3443, 3444, 3445, 3446, 3447, 3448, 3449, 3450, 3451, 3452, 3453, 3454, 3455, 3456, 3457, 3458, 3459, 3460, 3461, 3462, 3463, 3464, 3465, 3466, 3467, 3468, 3469, 3470, 3471, 3472, 3473, 3474, 3475, 3476, 3477, 3478, 3479, 3480, 3481, 3482, 3483, 3484, 3485, 3486, 3487, 3488, 3489, 3490, 3491, 3492, 3493, 3494, 3495, 3496, 3497, 3498, 3499, 3500, 3501, 3502, 3503, 3504, 3505, 3506, 3507, 3508, 3509, 3510, 3511, 3512, 3513, 3514, 3515, 3516, 3517, 3518, 3519, 3520, 3521, 3522, 3523, 3524, 3525, 3526, 3527, 3528, 3529, 3530, 3531, 3532, 3533, 3534, 3535, 3536, 3537, 3538, 3539, 3540, 3541, 3542, 3543, 3544, 3545, 3546, 3547, 3548, 3549, 3550, 3551, 3552, 3553, 3554, 3555, 3556, 3557, 3558, 3559, 3560, 3561, 3562, 3563, 3564, 3565, 3566, 3567, 3568, 3569, 3570, 3571, 3572, 3573, 3574, 3575, 3576, 3577, 3578, 3579, 3580, 3581, 3582, 3583, 3584, 3585, 3586, 3587, 3588, 3589, 3590, 3591, 3592, 3593, 3594, 3595, 3596, 3597, 3598, 3599, 3600, 3601, 3602, 3603, 3604, 3605, 3606, 3607, 3608, 3609, 3610, 3611, 3612, 3613, 3614, 3615, 3616, 3617, 3618, 3619, 3620, 3621, 3622, 3623, 3624, 3625, 3626, 3627, 3628, 3629, 3630, 3631, 3632, 3633, 3634, 3635, 3636, 3637, 3638, 3639, 3640, 3641, 3642, 3643, 3644, 3645, 3646, 3647, 3648, 3649, 3650, 3651, 3652, 3653, 3654, 3655, 3656, 3657, 3658, 3659, 3660, 3661, 3662, 3663, 3664, 3665, 3666, 3667, 3668, 3669, 3670, 3671, 3672, 3673, 3674, 3675, 3676, 3677, 3678, 3679, 3680, 3681, 3682, 3683, 3684, 3685, 3686, 3687, 3688, 3689, 3690, 3691, 3692, 3693, 3694, 3695, 3696, 3697, 3698, 3699, 3700, 3701, 3702, 3703, 3704, 3705, 3706, 3707, 3708, 3709, 3710, 3711, 3712, 3713, 3714, 3715, 3716, 3717, 3718, 3719, 3720, 3721, 3722, 3723, 3724, 3725, 3726, 3727, 3728, 3729, 3730, 3731, 3732, 3733, 3734, 3735, 3736, 3737, 3738, 3739, 3740, 3741, 3742, 3743, 3744, 3745, 3746, 3747, 3748, 3749, 3750, 3751, 3752, 3753, 3754, 3755, 3756, 3757, 3758, 3759, 3760, 3761, 3762, 3763, 3764, 3765, 3766, 3767, 3768, 3

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## LOW-TEMPERATURE PRESERVATION OF FOODS

the effect of freezing rate on the textural quality of vegetables. Woodruff [110] indicated that a short interval between the initial penetration of ice crystals and complete freezing was essential for the preservation of natural attractiveness of vegetables. He recommended a rate of ice formation of 0.1 cm/min. Several investigators have reported that the firmness of blanched asparagus spears was retained to a higher degree by rapid freezing than by slow freezing [58-66, 69, 70]. Lee and Johansson [61], using a cryostat and a texture panel to evaluate the texture of cooked asparagus, concluded that rapid freezing of blanched asparagus spears by immersion in liquid nitrogen or sugar syrup at  $-17.8^{\circ}\text{C}$  was more effective for maintaining tissue firmness than slow freezing in moving air at  $-45.6^{\circ}\text{C}$ . Juriya and March [62] found less drip loss asparagus frozen rapidly in dry ice than from asparagus frozen at  $-18^{\circ}\text{C}$ .

In a study of blanched peas and green beans frozen at five maximum rates ranging from rapid (liquid air) to very slow (frozen in an insulated box at either  $-21.6$  or  $-37.8^{\circ}\text{C}$ ), Lee et al. [60] found a significant difference in texture only between peas and beans frozen in liquid air and those frozen at slower rates. All samples were stored for six months at  $-18^{\circ}\text{C}$  prior to evaluation. The rapidly frozen vegetables were much softer than the vegetables frozen at the slower rates. These investigators attributed the softness of the rapidly frozen products to tissue cracking during freezing.

Brown [17] indicated that, if green beans were held in liquid nitrogen after being "completely frozen," the rapid but nonuniform temperature drop causes cracking. To avoid cracking, the vegetable pieces should be slow cooled and should be removed from the liquid nitrogen within 2 sec after "complete" freezing [12, 130]. Freezing precautions to avoid cracking, Wilford and Brown [130] and Brown [12] examined the benefits of rapidly freezing blanched green beans (stored at  $-20.0^{\circ}\text{C}$ ) from the standpoint of texture. These investigators found that cooked beans previously frozen in liquid nitrogen had a firmer texture (measured by Warner shear press and panel) than beans frozen individually in a wire tray in air blast at temperatures between  $-8.7$  and  $-40^{\circ}\text{C}$  or beans frozen in half-pint canisters at  $-28.9^{\circ}\text{C}$ . According to Brown [12], the difference in texture between beans frozen at  $-4.7^{\circ}\text{C}$  (slow freezing) and those frozen at  $-40^{\circ}\text{C}$  (fast freezing) was just noticeable. Panel analysis indicated that cooked beans previously frozen at subatmospheric slow freezing temperatures were

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slightly and somewhat rubbery, whereas the texture of the beans frozen in liquid nitrogen was closer to that of fresh beans [12]. Prolonged soaking (10 min at about  $180^{\circ}\text{C}$ ) reduced the textural differences between the rapidly frozen and slowly frozen beans. Recently Satchell [11] also reported that green beans frozen in liquid nitrogen, in spite of some cracking, were superior in texture and water-holding ability to those frozen in moving air at  $-40^{\circ}\text{C}$  or still air at  $-18^{\circ}\text{C}$  (Fig. 7-5). The beans in this study were stored for only one day.

Lee and Carman [59] studied frozen pea beans and corn and were unable to detect textural differences between rapidly frozen and slowly frozen samples.

During the slow freezing of fruit tissues, intercellular ice formation can damage the cell walls and middle lamellae to such an extent that the thawed product is much softer than the fresh fruit. Goldschmidt [42] showed that the pectin source for the texture of thawed strawberries previously frozen at  $-18^{\circ}\text{C}$  (and stored for one day) was much lower than that for the fresh fruit. However, the force required to crush the fresh strawberries was about twice that required for the slowly frozen product (Fig. 7-5). The large amount of drip (about 40% of the total weight) which occurred during thawing of the slowly frozen strawberries was an additional indication of the damage incurred during the freezing process. Bevilacqua [103] reported that the firmness of raw apples as measured by a B. F. Texturemeter was reduced to about one-fourth the original value by freezing the tissue at  $-10^{\circ}\text{C}$  (slow freezing) followed by immediate thawing.

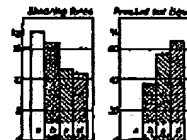
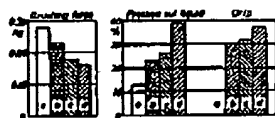


Fig. 7-5. Quality assessment of fresh and frozen green beans. (a) Fresh; (b) frozen in liquid nitrogen; (c) frozen quickly at  $-40^{\circ}\text{C}$ ; (d) frozen slowly at  $-18^{\circ}\text{C}$ . (From Carman, Lee, et al. [59]; courtesy of Pergamon Press.)



Since intercalation too should not damage the cell walls or permeability to any appreciable extent, rapid freezing should be advantageous in maintaining the seasonal quality of fruit tissues. Indeed Ouchibault [42] stated that strawberries frozen slowly in still air at  $-13^{\circ}$  were inferior in texture to strawberries frozen in moving air at  $-40^{\circ}$ , or in liquid nitrogen. However the forces required to crush the third samples are considerably greater for the rapidly frozen strawberries than for those frozen at  $-13^{\circ}$ . According to Bruneau [41], *vacuum* fruits such as strawberries and apples do not crack as much during rapid freezing as do biennial vegetables, such as green beans. However, Sterling [10] reported that apple tissues cracked extremely early during freezing in liquid nitrogen, so that the resistance of the third product was much less than that of the first product.

Very little information is available regarding the effect of chlorine bearing groups on the strength and general quality of fibers. When triazine and acetyl triazine in 50% aqueous form were held in acetone as "d-d", the strength was measured by a random-tensile instrument rapidly during the first five weeks and then lowered off gradually during the next five weeks [6]. Fibers of thymid and thymid chlorides were found to combine very well with organopolysiloxane coatings of chlorides in both sides. One and McCarty [2] demonstrated that the presence of a chlorine atom in the main chain of a polymer was related to the decomposition of pyrolysis in the early limits of chlorine. As the number of free chlorine groups increase in the pyrolytic materials, more metallic bridges form between the polymers and this enhances their cohesion.

### 2-2-1 Prospective Agents

Although considerable research has been carried out on cryoprotection for living cells, little information is available on chemicals which actively protect the functional quality of fruits and vegetable tissues during freezing, storage, and thawing. Bunting [101] found that the texture of apple tissue frozen at  $-17^{\circ}\text{C}$  can be protected to some extent in solutions containing either glycerol, ethylene glycol, propylene glycol, sorbitol, sucrose, or sodium chloride. The firmness of the frozen-thawed apple tissue was maintained better by increasing the concentration of the polyhydric cryoprotectors from 1 to 3M. According to Bunting [102], the protective role of the polyhydric cryoprotectors involved a restriction of ice crystal size in the intercellular spaces. Solids glycols was particularly effective in maintaining the firmness of frozen apple tissue, but was less effective for peaches and strawberries. According to Bunting [101], the salt effect on the texture of apple tissue can be attributed to (1) accumulation of acids between cells, (2) migration of salt ions which to increase turgidity and (3) compatibility of the cell walls. The texture of frozen berries also can be improved by adding an anhydrous organic solids to the immersion syrup [12, 116].

#### 7-4 CHEMICAL CHANGES IN PLANT TISSUES DURING FREEZING AND FROZEN STORAGE

During frozen storage at temperatures of  $-2^{\circ}$  or lower, chemical growth stages [75] but chemical changes in food phytochemicals may occur even at  $-10^{\circ}$ , the temperature which is commonly used for the chemical storage of frozen fruits and vegetables [24, 37, 41, 51, 52, 57]. The major chemical changes which can be important in quality deterioration of frozen fruits and vegetables are: (1) reactions associated with the development of off-odors and off-flavors, (2) pigment degradation, (3) enzyme browning, and (4) autooxidation of ascorbic acid. Attention of the pH of glass vials during freezing and frozen storage may be linked with the onset of these reactions and with deterioration of quality. Microbiological control of chemical deterioration in frozen phytochemicals can be achieved in most instances by prefreezing commensals such as browning or enzyme, or by decreasing the storage temperature to  $-15^{\circ}$  or lower.

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## 7-4.1 REACTIONS ASSOCIATED WITH THE DEVELOPMENT OF OFF-ODORS AND OFF-FLAVORS

During frozen storage of unblanched or underblanched vegetables, volatile compounds such as esters, aldehydes, ketones, and alcohols (volatile products of glycolysis) accumulate in the tissue and this accumulation coincides with the development of off-odors [48, 51, 52]. These off-odors appear even after cooking, and may be considered broadly as resembling the odors of:

- (1) village or aged cheese (acetaldehyde, acetone, acetoin, and acetal).
- (2) alfalfa (green and green beans), (3) aspartic acid (green beans),
- (4) aspartic acid, (5) aspartic acid (green beans), and
- (6) aspartic acid (green beans) [53].

Bedford and Jeeley [4] were the first to report that volatile aldehydes were formed in raw and underblanched plant tissues (green beans) during frozen storage. They found that the acetaldehyde content of frozen beans decreased and the organoleptic acceptability of the cooked product improved as the duration and temperature of blanching were increased. Later studies showed that acetaldehyde accumulated in other unblanched or underblanched vegetables such as brussels sprouts, peas, lima beans, and squash during frozen storage [49, 51]. Although acetaldehyde is not the only volatile aldehyde in the off-odor of frozen vegetables, the compound present in some frozen green beans correlated with the intensity of off-odor [51, 49, 52]. With some frozen vegetables, investigators [52] have failed to find a correlation between acetaldehyde and off-odors. Both Jeeley and Bedford [4] found that the chemical content of frozen brussels sprouts was related to the degree of off-odor.

The enzymes responsible for the production of acetaldehyde and ethanol in frozen green beans are inactivated at a lower blanching temperature than enzymes involved in the development of off-odors [52]. These investigators also found that, at a storage temperature of  $-12.2^{\circ}\text{C}$ , formation of acetaldehyde and ethanol in underblanched green beans was inhibited almost completely, yet off-odors developed progressively.

Off-odors in unblanched and underblanched frozen vegetables may be caused by enzymic oxidation of lipids. This lipid degradation is possible since consideration is given to the types and amount of lipids in vegetable tissue. The crude lipid content of vegetables, on a dry weight basis,

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ranges between 1.25 and 3.3% [54]. According to Lee and Hurrell [63], the crude lipid contained in green beans with a chloroform-methanol extract, consisted of phospholipids, neutral fat, and fatty acids. Substrates (such as linoleic and linolenic acids) of lipase activity have been found in peas by Lee and Hurrell [62]. According to Lee and Hurrell [63], high peroxide and acid numbers were obtained with lipid from unblanched peas held at  $-10^{\circ}\text{C}$  for five years. On the other hand, lipid from blanched peas held under the same freezing conditions had peroxide numbers of zero. Furthermore, Lee et al. [64] reported that off-flavors developed in frozen unblanched peas, corn, and snap beans held at  $-10^{\circ}\text{C}$  for up to four weeks. However, the peroxide and acid numbers of the lipids from these unblanched vegetables increased with frozen storage time. Figure 7-7 illustrates the magnitude of increases in peroxide values for unblanched frozen peas.

According to Lee and Hurrell [64], oxidative rancidity is not the main cause of flavor deterioration in frozen blanching peas. The highest chlorophyllase half (70%) activity observed in raw peas after 40 days of storage at  $-10^{\circ}\text{C}$  was much below the threshold value for oxidative rancidity. Further, the odor of the frozen raw peas was much different from the rancid odor in blanching raw peas stored with a high pH number.

Lipase activity may play an important role in the oxidation of lipids

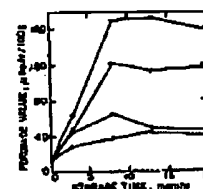


Fig. 7-7. Peroxide values of frozen unblanched peas. a, stored in oxygen at  $-2.2^{\circ}\text{C}$ ; b, stored in nitrogen at  $-2.2^{\circ}\text{C}$ ; c, stored in nitrogen at  $-10^{\circ}\text{C}$ ; d, stored in nitrogen at  $-15^{\circ}\text{C}$ . (From Hurrell and Bedford, loc. cit. [63]; courtesy of the Society of Chemical Industry, London.)

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during the frozen storage of unblanched and inadequately blanched vegetables [110, 112]. Lipoygenase catalyzed oxidation of linoleic and linolenic acids to hydroperoxides which decompose to carbonyl compounds [103]. Gas chromatographic analyses by Baugmann and Arnold [5] on the volatile compounds from frozen-blended raw peas revealed the presence of hexanal, an oxidation product of lipoygenase action on polyunsaturated fatty acids.

Shaw and Morris [63] reported lipoygenase was present in a wide variety of fruits and vegetables. Detailed studies have been conducted on lipoygenase in peas and sweet corn [69, 109-111]. According to Shaw and Morris [63], lipoygenase activity was quite high in frozen raw peas at  $-20^{\circ}\text{C}$ . They showed an increase in the TBA value of lipids in frozen peas to frozen storage time progressed to 106 days. The peroxide value of lipids extracted from frozen raw peas and from underblanched commercial peas also increased after extended periods of frozen storage [109, 110]. Studies on the 199S isozyme in unblanched frozen peas at  $-0.4$  and  $-22.2^{\circ}\text{C}$  were studied by Smith and Spivey [16]. The thiobarbituric acid values increased continuously throughout the 18-month storage period but the peroxide values increased only during the first eight months and thereafter leveled off or decreased slightly. The TBA values also indicated that the extent of lipid oxidation was greater at the higher storage temperature ( $-0.4^{\circ}\text{C}$ ), at any given time within the 18-month period.

#### 3-4.2 Chlorophyll Deterioration

During frozen storage of blanched green vegetables such as peas, green beans, and spinach at  $-18^{\circ}\text{C}$  or above, the bright green color of the recently frozen product slowly changes to grayish-green, then to olive green, and subsequently to brownish green [21-24]. However, in unblanched green vegetables, the rate of color change is much more rapid. Such color changes have been attributed to the conversion of chlorophyll *a* and *b* to the corresponding brown pheophytins [17, 70].

Dietrich et al. [34] reported that when the difference in chlorophyll content of the green bean samples exceeded approximately 4%, then a color difference was visually detectable by about 25% of an evaluating panel. With frozen peas, a difference of about 1.1% in chlorophyll content can be detected visually by 75 to 84% of the total panel judged [11]. Thus it is important to avoid degradation of even small amounts of chlorophyll.

Blanching of green vegetables is essential for inhibiting chlorophyll conversion to pheophytins during frozen storage. Wagenvoort et al. [113] demonstrated that chlorophyll in frozen raw beans degraded rapidly. Walber [114] studied frozen unblanched green beans stored at  $-20^{\circ}\text{C}$  and found a negative straight-line relationship between storage time up to 20 days and chlorophyll retention. As the end of the 20-day period of frozen storage, about 45% of the chlorophyll in the unblanched beans was lost, whereas all of the chlorophyll in the blanched 15 green curved beans previously blanched at  $100^{\circ}\text{C}$  for 45 sec (from maximum to insensitive peroxide and catalase). According to Smith and Spivey [16], storage of unblanched peas for 18 months at  $-0.4$  and  $-22.2^{\circ}\text{C}$  resulted in chlorophyll conversions of 57.6 and 14.9%, respectively. On the other hand, with blanched peas (3 up to  $100^{\circ}\text{C}$ ), the conversion to pheophytin at  $-0.4$  and  $-22.2^{\circ}\text{C}$  was only 15.9 and 7.9%, respectively, for the 18-month period.

Although blanching is necessary to inhibit chlorophyll degradation during frozen storage, it is not a perfect method of control since blanching would cause a significant conversion of chlorophyll to pheophytin. The extent to which this occurs is dependent on factors such as the ratio of chlorophyll *a* and *b*, blanching time and temperature, and pH. The thermal stability of chlorophyll *a* is less than that of chlorophyll *b* [15]. The amount of chlorophyll conversion in vegetables is directly related to blanching temperature and time [25, 114], with blanching temperatures between  $87.8^{\circ}\text{C}$  and  $100^{\circ}\text{C}$  the rate of chlorophyll conversion in green beans varies from 4 to 16% in a blanching time of 1 to 5 min [25]. Blanching of green beans at a high temperature ( $83.5^{\circ}\text{C}$  to  $100^{\circ}\text{C}$ ) and a short time (1 to 2 min) causes less chlorophyll degradation than blanching at  $87.8^{\circ}\text{C}$  to  $100.5^{\circ}\text{C}$  for 3 to 5 min [25, 81]. Walber [114] reported that about 1% of the total chlorophyll was converted to pheophytin during blanching of beans for 45 sec at  $100^{\circ}\text{C}$ . Thus it is apparent that the amount of chlorophyll degraded during blanching is small in relation to that which is lost during frozen storage of an unblanched product.

The rate at which chlorophyll is converted to pheophytin is directly related during frozen storage is dependent on the amount of glucose damage prior to freezing. Dietrich et al. [34] found a negative straight line relationship between the per cent chlorophyll retained immediately after blanching and the rate of chlorophyll deterioration during frozen storage. Other studies by Dietrich et al. [35] substantiated these observations.

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Purchasing, the deterioration rate of chlorophyll in various foodstuffs (measured as the storage temperature is raised from  $-18.3$  to  $-3.0^{\circ}\text{C}$ ) (Fig. 7-4).

Chlorophyll degradation occurs very slowly in frozen foods at  $-18^{\circ}\text{C}$  and below. The difference in color between control foods (stored at  $-18.3$  or  $-30.0^{\circ}\text{C}$ ) and those stored at  $-10^{\circ}\text{C}$  first became evident in 75% of a panel after an average of 101 days of storage. On the other hand, only about eight days of storage were required for a perceptible color difference to develop between control samples at  $-18.3^{\circ}\text{C}$  and those stored at  $-10.0^{\circ}\text{C}$ .

The data in Table 7-1 illustrate that chlorophyll degradation at different rates in frozen peas, green beans, and spinach held at a specific storage temperature. Chlorophyll is converted to pheophytin at a much greater rate in frozen green beans than in frozen peas at  $80^{\circ}\text{F}$  (given temperature between  $-7$  and  $-10^{\circ}\text{C}$ ). Miller [12] reported that during a 12-

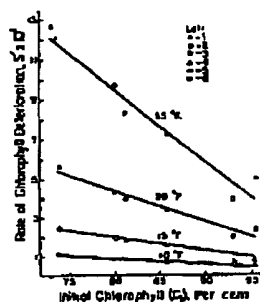


Fig. 7-4. Effect of initial chlorophyll level (C) on the rate of chlorophyll deterioration in frozen green beans at various storage temperatures. (From Dietrich et al., Ref. [24]; courtesy of the Institute of Food Technologists.)

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TABLE 7-1  
Frozen Storage Time (Months) Required for 10%  
Decrease in Chlorophyll Content of Selected Green Vegetables\*

Product	Storage Temperature		
	$-18^{\circ}\text{C}$	$-10^{\circ}\text{C}$	$-5^{\circ}\text{C}$
Peas	45	17	2.5
Spinach, leaf	30	6	1.6
Spinach, chopped	34	5	0.7
Green beans	20	2	0.7

\*Giam and Dietrich [20].

month storage period at  $-10^{\circ}\text{C}$ , the per cent chlorophyll converted to pheophytin was about 35 for frozen spinach, 40 for green beans, and 6 for peas.

Measurement of plant tissue can accelerate chlorophyll degradation. When spinach leaves are chopped prior to freezing, the rate of chlorophyll conversion to pheophytin is doubled during frozen storage [27].

According to Walker [13], conversion of chlorophyll to pheophytin is not the only cause of color loss in frozen foods. Apparently non-enzymatic oxidation of chlorophyll and pheophytin can also occur after 12 months of storage at  $-18^{\circ}\text{C}$ . Since a marked increase in the peroxide value of lipid in frozen blanched beans occurred after 12 months at  $-18^{\circ}\text{C}$ , and this coincided with significant oxidation of chlorophyll and pheophytin, it is likely that oxidation of these pigments involves free radicals formed during peroxidation of lipids. The blanching time at  $100^{\circ}\text{C}$  has a profound influence on the rate of chlorophyll oxidation of beans. Walker [14] found that the loss of chlorophyll by oxidation after 20 days storage at  $-18^{\circ}\text{C}$  was greatest in unblanched green beans, whereas beans blanched for 45 or 60 sec had no detectable loss of chlorophyll. However, as the blanching time increased above 60 sec, the rate of chlorophyll oxidation in the frozen beans increased after an initial lag period of a few days. The rapid oxidation of chlorophyll in unblanched beans and peas may be explained by the catalytic effects of lipoperoxide and a lipoperoxide breakdown factor found in some legumes [25, 26].

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#### 7.4.3 Oxygen-Catalyzed Oxidative Browning

When raw apples, peaches, pears, cherries, mushrooms, herbs, potatoes, and cauliflower are heated, stored, and sliced, undesirable brown pigments are formed. This reaction is of major concern since sensory evaluation of 13 different lots of commercially frozen peaches indicated that overall quality was directly related to the amount of browning (40). The browning of plant tissues is usually caused by enzymatic oxidation of phenolic compounds in the presence of oxygen (41, 42).

In pears and apples, chlorogenic acid and catechins are the major substrates of naturally occurring o-dihydroxy oxidases (polyphenoloxidases, phenolases, tyrosinases) (43). In potatoes, tyrosine can be readily oxidized in the presence of the naturally occurring o-dihydroxy oxidase. The optimum pH for o-dihydroxy oxidases is in the vicinity of 6 (44).

Disruption of native cells by low temperatures can initiate oxidative browning by facilitating contact between o-dihydroxy oxidase and its substrates. Browning of frozen plant tissues is usually more severe than the surface browning of fresh plant tissues in general at a higher level than that in the frozen tissues. However, improved as well as surface browning may take place in late harvest-tissue products such as apple slices when the numerous intercellular spaces generally contain some oxygen (22, 45).

Freeze distribution in frozen plant tissues can be obtained or prevented by: (1) better inspection of the samples, (2) addition of browning inhibitors such as sulfur dioxide and ascorbic acid, and (3) minimization of oxygen. Denaturation of o-dihydroxy oxidase in your tissues occurs slowly at temperatures between 50 and 60° and rapidly at temperatures of 80° and above (46). Inactivation of o-dihydroxy oxidase in apple slices can be achieved without a serious loss of quality by simply blanching prior to freezing (47).

Since blanching of plant tissues may reduce the concentration of desirable water-soluble compounds and perhaps promote development of undesirable chemical odors, discolored tissue in frozen fruits to be used for dessert purposes should be controlled by treatment with a browning inhibitor. Ascorbic acid and its salts are useful for inhibiting the activity of o-dihydroxy oxidase in frozen and thawed fruits and vegetables. According to Purdie (48), about 10 ppm of  $As_2$  is sufficient to inhibit o-dihydroxy oxidase in apples. For many fruits and vegetables,

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penetration of ascorbic acid just below the surface of tissue is sufficient since inhibition of surface browning is of primary concern. However, with apple slices, complete penetration to the center is considered to curtail internal browning. Frozen apple slices can be processed against enzymatic browning by dipping them in a solution containing 1000-1250 ppm  $As_2$  for 6 to 8 hr prior to freezing (49). Frozen slices treated as above contain about 100 ppm  $As_2$ .

Ascorbic acid has been used commercially for many years as an additive to sugar syrups of frozen and dried low-moisture surface browning (5, 5, 196). Concentrations of 150 to 250 mg of ascorbic acid per pound of finished product are usually adequate for this purpose. Goussard et al. (40) reported that the amount and intensity of browning of sliced fruit-thawed peaches were inversely related to ascorbic acid content. In their study, subjective measurement of browning consisted of counting the number of peach slices which had brown discoloration, whereas an objective evaluation of browning was made by obtaining Hunter L<sub>a</sub> (reflectance) and b (degree of yellowness) values. It is of importance to note that peach slices submerged first in ascorbic acid-containing syrup and then exposed to air at -6.7°, exhibited brown discoloration, within a few weeks. Peach slices that remained completely submerged in syrup at the same temperature did not change in color during a 45-day storage period. Coverage of the slices with sugar syrup (10-15%) significantly restricted oxygen migration into the peach slices.

Several studies have demonstrated the influence of oxygen content in the headspace on the rate of browning. According to Burdick et al. (41), red wax cherries discolored when they were exposed to air in the headspace during frozen storage for 50 days at -6.7°. These cherries were packed in a 20% sucrose syrup which did not contain ascorbic acid. The rate of browning in these frozen cherries was much lower when packed in hermetically-sealed tin cans rather than in metal-rim, paperboard-lined containers. However, if the storage temperature was maintained at -16° or lower, the type of container was not an important factor in the prevention of browning. In another study, Burdick and Stone (50) studied frozen peaches packed in sugar syrup containing ascorbic acid and showed that a vacuum of 15 in. Hg in the headspace of sealed cans prevented discoloration during a storage period of six weeks at -6.7°.

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At low concentrations, ascorbic acid does not inhibit the activity of  $\alpha$ -glycosyl oxidase but rather functions as a reducing agent to keep the oxidizable polyphenols in a reduced state [2, 31]. At high concentrations, the ascorbic acid retards the polyphenols in the reduced state until  $\alpha$ -glycosyl oxidase undergoes "enzyme inactivation" [32]. In tissues with an active  $\alpha$ -glycosyl oxidase system, ascorbic acid is gradually oxidized and eventually the polyphenolic compounds are oxidized. Thus it is not surprising that under adverse conditions of frozen storage, ascorbic acid is gradually lost and discoloration of tissues occurs.

Further discussion of methods of inhibiting enzymatic browning in fruits and vegetables is presented in Chapter 11.

## 7-4.4 Oxidation of Ascorbic Acid

In the presence of dissolved oxygen, ascorbic acid is  $\alpha$ -quinoxalination is oxidized to dehydroascorbic acid (DHA) which is in turn oxidized irreversibly to 2,3-dihydroxyacid and its degradation products [3]. The rate of ascorbic acid oxidation is directly proportional to the square root of the oxygen concentration and inversely proportional to the square root of the hydrogen ion concentration in acid solutions [33]. Ascorbate oxidase which naturally is very pure enzyme and if it is not inactivated, it will catalyze oxidation of ascorbic acid during the freezing process [3].

If fruits and vegetables are frozen in hermetically sealed metal containers with very low headspace oxygen, no appreciable amount of ascorbic acid will be oxidized. On the other hand, if fruits and vegetables are packaged in composite containers with metal paper walls and metal caps or in plastic-lined paper board boxes, the oxygen permeability is sufficient to cause substantial deterioration of ascorbic acid during frozen storage.

In addition to the factors cited above, the rate of ascorbic acid oxidation depends on the temperature of frozen storage and pH of the fruits and vegetables. As shown in Fig. 7-6, a high subfreezing temperature of  $-10^{\circ}\text{C}$  results in nearly complete oxidation of ascorbic acid in blanched green peas during a period of about one month [34]. In the same product stored at normal commercial temperatures of  $-18^{\circ}\text{C}$ , only about one-third of the total ascorbic acid was oxidized over a one-year period. Similar losses of ascorbic acid occur in beans and spinach.

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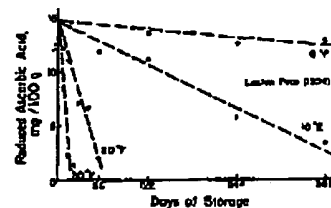


Fig. 7-6. Loss of reduced ascorbic acid in frozen peas at various storage temperatures. (From Elstrup et al., Ref. [35]; courtesy of the Institute of Food Technologists.)

As the pH is lowered, the stability of ascorbic acid in food generally improves. For example, the pH of strawberries is much lower than that of green vegetables and as a consequence, ascorbic acid in strawberries at  $-18^{\circ}\text{C}$  is stable for at least one year [32]. When strawberries are stored at a temperature of  $-12^{\circ}\text{C}$ , about 50% of the original ascorbic acid is oxidized in about 100 days, but this loss is much lower than that in most green vegetables stored under similar conditions.

In some instances, the rate of oxidation of ascorbic acid in frozen systems either increases or decreases less than expected. The circumstances and causes are discussed in Sec. 8-6.

## 7-4.5 Changes in pH

During the freezing of plant tissues, inorganic and organic salts are concentrated in the unfrozen phase. As the freezing temperature decreases, salts are precipitated out of the unfrozen phase at their solubility limits and as a consequence the pH of the unfrozen phase may change. van den Berg [6] and van den Berg and Bess [9] studied pH changes that occur during the freezing of various phosphate buffers. Some of their data are reported in Chap. 8 (Sec. 8-3.3). Fruits and vegetables also undergo changes in pH during freezing. van den Berg [7, 8] studied peas stored at  $-10^{\circ}\text{C}$  and observed that the pH dropped from 6.7 to about 6.0 during the first three days, increased to 7.0 after

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Fig the milk rose to three weeks and then dropped to 6.4, where it remained to the end of the 75-day storage period. When green beans and cauliflower were frozen on -10°, their pH values dropped to about 4 and 4.8, respectively, during the first 10 days of storage. After a few weeks, pH values increased to about 6. Storage of beefsteak cutlets at -10° did not alter the above-mentioned pattern of pH change during storage. von den Berg [7] suggested that the first pH decrease during frozen storage of vegetables was caused by precipitation of alkaline salts, potassium, and sodium phosphates. Precipitation of acid potassium phosphate, and sodium and potassium chloride were considered to be responsible for subsequent increases in pH. So far, no studies have shown a relationship between pH change in food phytochemicals and quality deterioration during frozen storage. However, it may be speculated that even with a small change in pH, enzyme activity would be altered and cell membranes would be damaged.

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